IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re-application of
Aguera et al.

Group art Unit : 1636
Examiner : Akhavan, Ramin

Serial N° : 09/986,632
Filed : 11/09/2001

DECLARATION UNDER RULE 132

Hon. Commissioner of Patents and Trademarks
WASHINGTON D.C. 20231

Sir :

I, Marie-Françoise Belin, French citizen, having a Ph. D (Doctor es Sciences) in Neurosciences, and over 15 years of experience of group leadership in Research Laboratory at INSERM in Neurobiology and neuroinflammation, with 120 publications,
declare :

I am the inventor of the present application and I am aware of the fact that the Examiner has rejected the claims for lack of enablement.

The following experiments have been made under my personal supervision:

CRMP1-deficient mice have been produced and were made to develop experimental allergic encephalomyelitis (EAE) an experimental model of MS.

MATERIALS AND METHOD :

1) Construction of targeting vector and screening for targeted ES clones.

The CRMP1 genomic fragments were isolated from R1 ES cell DNA by PCR amplification. CRMP1 specific primers with restriction site overhangs, derived from the CRMP1 cDNA sequence, were used to amplify 3rd and 4th introns with adjacent exon sequences:

1.CCGCGGCCGCTGGAGGCGAATGGCCGA 3^{rd} exon, forward, Not1 site

2.GCGTCTAGATTTCTCAAAGGAAGTCAACAAGCTG 4^{th} exon, reverse, Xba1 site

3.GCGGGATCCGTATGATGGTGTTCGGGAAG 4^{th} exon, forward, BamH1 site

4.CGGCGCGCCGTCGACAGACATCTGGTACAGGTC 5^{th} exon, reverse, Sal1 site

The primer pair 1 and 2 amplified 5kb fragment corresponding to the 3^{rd} intron and the pair 3 and 4 amplified a 3kb segment representing intron 4. Sequencing of the PCR products confirmed their identity and revealed the predicted exon/intron functions

The 5 kb and 3.5 kb, 5' and 3' CRMP1 fragments, were then inserted into the pIRES-LacZ-neo (cassette positioned such that it would disrupt and delete an internal part of the 4^{th} exon of CRMP1 gene). HSV TK gene was added to the 3' end of the construct as a negative selection marker. Not1 linearized vector, (25 µg), was electroporated (240V, 500 µF) into 2 x 107 R1 ES cells (Nagy et al., 1993). The ES cells were selected with 150 µg/ml G418 (active form) and 2 nM GANC. DNA of individual ES colonies was screened by PCR with a forward neo primer: neo5 (primer d): TATCGCCTTCTTGACGAGTTCTTCTGA) and a primer from 5th exon outside of the homologous region used in the vector (primer f): TCACCTGGCTGTCAGACATC. PCR conditions consisted of 36 cycles of 94°C for 20 sec, 60°C for 40 sec, and 72°C for 3min 30 sec using BRL Taq polymerase. The recombination was reconfirmed on the 3' end using primer neo5 (primer d) and a reverse primer from the 6th exon (primer g): TTTCTGCATGGACTAAGATCA, and on the 5' end using a forward primer from the 3rd exon (primer a): CAGAGGTGGACGCATCA) and a reverse primer from the IRES sequence (primer b): GGGCGGAATTCTCTAGCTAGA).

2) Generation of CRMP1-deficient mice.

Targeted ES cells were aggregated with ICR morula and implanted into pseudopregnant females. Male chimeras were mated initially with ICR females to screen for germ-line transmission. A transmitting chimeric male was then bred with 129SvE females and

their progeny was genotyped with primers derived form the neo gene: neo as (n2) and neo s (n1) sequences CCGACCTGTCCGGTGCCCTGAATGAA and TACCGTAAAGCACGAGGAAGCGGTCA, respectively). Homozygous mutant mice were obtained by intercrossing the heterozygotes and screening the progeny with primers neos + neoas (PCR conditions: 36 cycles of 94°C for 25s, 60°C for 40s, and 72°C for 1min) and U3s (primer c), derived from the portion of the 4th exon deleted in the targeted allele (CGAAGCAGCAGACACCAAATC) and U3as (primer e), reverse primer from the 4th intron (TGAAACAGGAGTGCATCCTTC) (PCR conditions). These two sets of primers were then used for all further genotyping.

3) Induction of EAE

The CRMP1-deficient mice were injected with MOG or bone marrow, in order to induce experimental allergic encephalomyelitis (EAE), a classic animal model for Multiple Sclerosis.

RESULTS :

Compared to control mice that were also injected with MOG or bone marrow and developed EAE, the CRMP1-deficient mice shown the first clinical symptoms of EAE more than 12 days later than in the control mice. The symptoms were attenuated and disappeared quicker than in the control mice.

In addition the CRMP 2 expression is upregulated while CRMP 5 is downregulated in animals with EAE compared to healthy untreated animals. The modulation of CRMP during demyelination in an experimental model of MS indicates the involvement of CRMP in demyelination.

In light of the above results, it is apparent that the modulation of CRMPs is of great interest for the treatment of myelin disorder.

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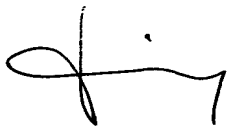
The undersigned Declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this August day of 18th 2004

>MF BELIN



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Hon. Commissioner of Patents and Trademarks
WASHINGTON D.C. 20231

Sir :
I, Bernard Zalc, French citizen, physician and having a Ph. D degree in Neurosciences (Biography attached),
declare :

I was asked to review U.S. patent application serial number 09/986,632 and to give my comments as to the invention proposed.

Multiple Sclerosis is the most frequent neurological disease of the young adult and the first cause of handicap after road accidents (car and motorcycle). With an incidence of about 1/1000, it is estimated that 250 000 patients in the US and 350 000 in Europe are suffering the disease. MS is characterized by the association of both inflammatory and demyelination components. Since inflammation is labile, it may explain the mode of evolution of the disease, with boots and remission. Demyelination, however, together with axonal loss is the cause of the permanent handicap. During the past 10 years, the introduction of immunomodulatory therapies (beta-interferon and glatiramer acetate) has successfully lowered the

burden of the inflammation component of the disease. These treatments impressively reduce the number of gadolinium enhanced lesions seen by MRI, and slightly reduce the number of relapses. It is noteworthy, however, that compared to the natural course of the disease the first reported follow-up of patients after 10 years of treatment has not shown any evidence of clinical improvement on the EDSS clinical score. This is somewhat disappointing. More worrisome are the finding by several groups that using experimental demyelinating models there seem to be a beneficial effect of the inflammatory reaction on the extent and rapidity of remyelination, suggesting that fighting the inflammation may, after all, not be such a good strategy in MS.

On the other hand, it is clear that it is the demyelination component, which is the cause of the permanent handicap in MS. In addition, the increase in the number of demyelinated lesions (and their extension) is responsible for the exacerbation of the handicap over the course of the disease. As a consequence, remyelination should be viewed as the key solution and future of therapeutic strategies in MS.

It is from this perspective that we need to examine the finding by the inventors that CRMPs are key players in the transduction of signals acting on oligodendrocytes, the myelin forming cells in the CNS.

Data from the inventors illustrate the involvement of CRMP-mediated signalization at the time of myelin formation. Myelin acts as an insulator of axons and allows the rapid propagation of the nerve impulse. Demyelination most often results in a conduction block, hence the handicap faced by MS patients. It is therefore easy to understand why the reestablishment of a myelin sheath around demyelinated axons (remyelination) is crucial to reestablish normal nerve conduction. CRMP appears as the cross road of several different signalization pathways, which are activated at the time of myelin deposition, i.e., at the time during development when oligodendrocyte processes wrap around the axon to generate the myelin sheath. As a result, CRMPs is a new target for increasing myelin formation and thus remyelination.

For all these reasons I consider the modulation of CRMPs proposed by the inventors, as a new therapeutic pathway for the prevention and treatment of myelin disorders. The forthcoming clinical trials are hence very promising.

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The undersigned Declarant declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true ; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed this 26 day of July 2004

Dr. Bernard ZALC
Directeur
INSERM U. 495

BIOGRAPHICAL SKETCH

NAME	TITLE
Bernard Zalc	Dr

CURRENT POSITION

Director of Research INSERM
Director of INSERM Unit 495

INSTITUTION AND LOCATION	DEGREE	YEAR	FIELD OF STUDY
CHU Pitié-Salpêtrière, Paris	MD	1974	Medicine
University Pierre & Marie Curie (Paris)	Dr. es Sciences	1981	Natural Science

Experience:

1972-1974: Graduate student, Neurochemistry Lab (Dr. N. Baumann), Hôpital de la Salpêtrière
1974-1975: Post-Doctoral fellow, Neuroscience Lab (Dr. N.S. Radin), U. of Michigan (USA)
1975-1976: Military National Service
1976-1978: Stagiaire of Research INSERM
1979-1980: Attaché of Research INSERM
1981-1988: Chargé of Research INSERM
1988- 2001: Director of Research INSERM 2ème Classe
2002- present : Directeur of Research INSERM 1ère Classe
1998- present : Director of INSERM Unit -495 "Biologie des Interactions Neurones-Glie".

Honours & professional positions:

Professional Services
University:
1981- 1988: Member of the Scientific Council of UFR Pitié -Salpêtrière
1984- 1988: Member of Conseil d'Administration of UFR Pitié-Salpêtrière
1995- 1998: Member of Scientific Council University Marnes la Vallée
1998- 2006: Member of the Scientific Council of UFR Pitié -Salpêtrière
National
1990-1994: Member of INSERM Neuroscience Study Section
1999- 2002: Member of the Scientific Council of INSERM
Organisation of Scientific Meetings:
1993: Chaiman of the ISN satellite meeting: "Emerging concept in Myelin Biology"
1998: Co-Chair (with B. Trapp) of the Gordon Research Conference: "Myelin"
2002: Vice-Chair (elected) of the Gordon Research Conference: "Myelin"
2004: Chair (elected) of the Gordon Research Conference: "Myelin"
Editorial Boards
-Journal of Molecular Neuroscience (1988-1992)
-Journal of Neurochemistry (1991-)
-Neurochemical Research (1992-1999)
-Journal Neuroscience Research (2000-)
-Neuron Glia Biology (2003-)

Current Research Group
Assistant Professor; Jean-leon Thomas (University); *Chargé of Research;* Nathalie Spassky (INSERM) *Post docs:* Maria Jose Barralobre (FRM), *PhDs*: Barbara Le Bras (FRM), Delphine Delaunay (MENRT) Elli Chatzopoullou (MENRT), *Research Assistant:* Katharina Heydon (EEC)

Biographical sketch:
Dr. Bernard Zalc has obtained his MD from Pitié-Salpêtrière Medical School in Paris (1974), and his PhD (Doctorate Es Sciences Naturelles) from University Pierre et Marie Curie (Paris). After graduating, Dr. Zalc has been a post-doc with Pr. Norman Radin at University of Michigan (USA)(1974-75) and then with Dr. P. Dupouey at the Institute Pasteur in Paris (1975-1976). Then Dr. Zalc received a position in Dr. Nicole Baumann's lab at INSERM (Institut National de la Santé et de la Recherche Médicale). In 1988 he was recruited as Directeur de Recherche at INSERM. Since 1998, Dr. Zalc is directing the INSERM Unit 495: Biologie des Interactions Neurones/Glie, which is located in Paris at the Hôpital de la Salpêtrière. Dr. Zalc's scientific interest is in the biology of myelin forming cells and related diseases, with a special focus on Multiple Sclerosis. For the past few years, he has concentrated his effort on the understanding of the origin during development of the oligodendrocyte, the myelin-forming cell in the central nervous system. Major findings from Dr. Zalc have been to establish the map of the restricted site of emergence of oligodendrocytes along the neural tube and to demonstrate that oligodendrocytes are generated by different sources of progenitors. His present work is devoted to the phylogenic and ontogenetic origin of myelin forming cells. Dr. Zalc's ongoing research is aimed at deciphering the molecular mechanisms that underlies the specification of a multipotent stem cell into an oligodendrocyte and on the molecules that control the migration of oligodendrocyte precursors.

Relevant publications in the last five years:

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PEREZ-VILLEGAS EM., OLIVIER C., SPASSKY N., PONCET C., COCHARD P., ZALC B, THOMAS J-L., MARTINEZ S. (1999) Early specification of oligodendrocytes in the chick embryonic brain. (1999) *Dev. Biol.* 216:98-113.

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